TRANSFECTION **CHROMATIDS** CENTROMERE KINETOCHORE **TELOMERE** ACM8 AND AgtWESneo DNA INTEGRATION TELOMERE В 3.34.4.57 **EUCHROMATIN** HETEROCHROMATIN (SATELLITE DNA) MOUSE CHROMOSOME (LMTK CELL LINE) AMPLIFICATION CENTROMERE FORMATION neo-CENTROMERE 12 Jan 20 19 18 SELECTION (G-418) D TELOMERIC DNA **KINETOCHORE** SATELLITE DNA MITOTIC SPINDLE DICENTRIC CHROMOSOME (EC3/7 CELL LINE) CHROMOSOME **BREAKAGE** "FOREIGN" DNA SATELLITE DNA neo-CENTROMERE TELOMERE FORMERLY DICENTRIC CHROMOSOME CHROMOSOME FRAGMENT WITH neo-CENTROMERE 10-15Mb (EC3/7 CELL LINE) **DUPLICATION-**SINGLE CELL CLONING G reo-CENTROMERE TELOMERE TELOMERE/mCPE 1.51 SATELLITE DNA SATELLITE DNA "FOREIGN"DNA 20-30Mb neo-MINICHROMOSOME (EC3/7-C5;EC3/7-C6 CELL LINES)

HELLER EHRMAN WHILE & MICAULIFFE LLP Sheet 1 of 5 e: ARTIFICIAL CHROMOSOMES, USES THEREOF AND THODS FOR PREPARING ARTIFICIAL CHROMOSOMES

Filed: April 17, 2001 .

Art Unit: 1632

Serial No.: 09/836,911

Examiner: Shukla, R. Our Docket No.: 24601-4021

MAR 1 3 2003

FIG. 1

TECH CENTER 1600/290

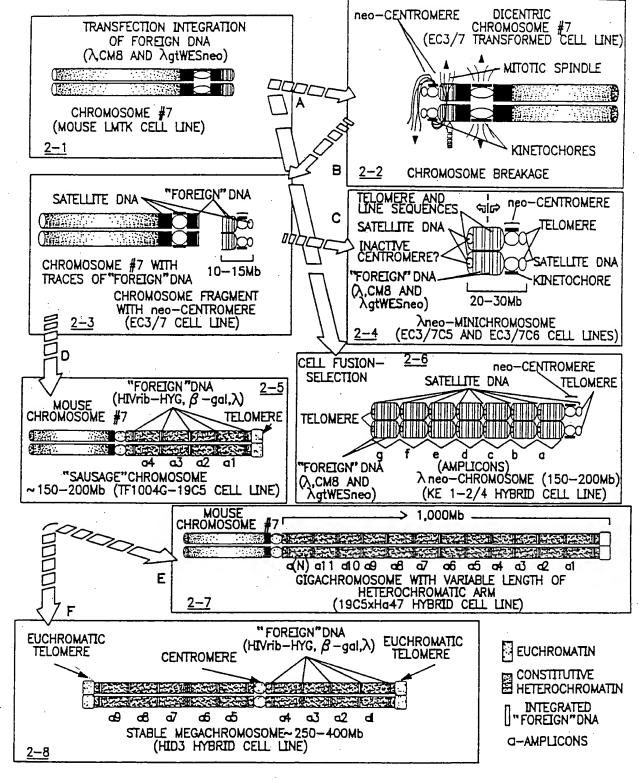


FIG. 2

TECH CENTER 1600/2900

HELLER EHRMAN WHIT E & MCAULIFFE LLP

Applicant: Hadlaczky et al. Serial No.: 09/836,911 Filed: April 17, 2001

Examiner: Shukla, R. Our Docket No.: 24601-4021

PRIMARY REPLICATION INITIATION SITE (MEGAREPLICATOR)

SECONDARY ORIGINS OF REPLICATION

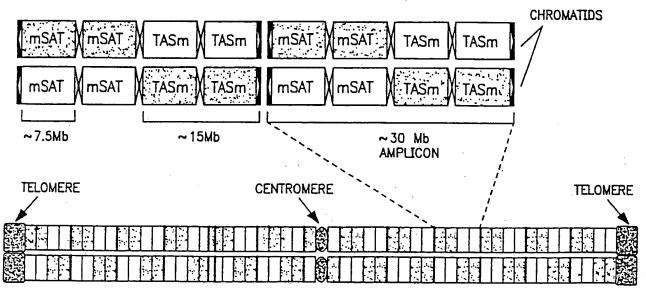
MEGAREPLICON OF THE CENTROMERIC REGION OF MOUSE CHROMOSOMES WITH TWO~7.5Mb TANDEM BLOCKS OF MOUSE MAJOR SATELLITE DNA (mSAT) FLANKED BY NON-SATELLITE DNA SEQUENCES

INTEGRATION OF"FOREIGN"DNA (pH132, pCH110,λ)

REPLICATION ERROR GENERATES INVERTED MEGAREPLICONS

TASm TASm

AMPLIFICATION PRODUCES A TANDEM ARRAY OF IDENTICAL CHROMOSOME SEGMENTS (AMPLICONS) THAT CONTAIN TWO INVERTED MEGAREPLICONS BORDERED BY THE HETEROLOGOUS ("FOREIGN") DNA



STABLE MEGACHROMOSOME (~250-400Mb)

FIG. 3

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Serial No.: 09/836,911 Examiner: Shukla, R.

Filed: April 17, 2001 . Art Unit: 1632

OUR DOCKET NO.: 24601-4021

EC3/7

MOUSE LMTK" (FIBROBLAST CELL LINE WITH neo-CENTROMERE)

SINGLE-CELL SUBCLONING

FUSION WITH CHO K20 CELLS AND 8 ▼ SELECTION WITH G418 AND HAT

EC3/7C5

MOUSE LMTK FIBROBLAST CELL LINE WITH THE neo-MINICHROMOSOME AND THE FORMERLY DICENTRIC CHROMOSOME

KE1 - 2/4

MOUSE-HAMSTER HYBRID CELL LINE WITH THE STABLE Aneo-CHROMOSOME

COTRANSFECTION WITH PLASMIDS pH132 (ANTI-HIV RIBOZYME AND HYGROMYCIN-RESISTANCE GENES), pCH110 (lacZ GENE) AND Act 875 Sam7 (λPHAGE), SELECTION WITH HYGROMYCIN Β

TF1004G-19C5

MOUSE LATK "FIBROBLAST CELL LINE WITH neo-MINICHROMSOME AND STABLE SAUSAGE CHROMOSOME - FUSION WITH CHINESE HAMSTER OVARY CELLS (CHO K20 CELL LINE), SELECTION WITH HAT

AND HYGROMYCIN B. 19C5xHa4, | 19C5xHa3|--RECLONING -- 19C5xHa47 (CARRIES THE GIGACHROMSOME)

MOUSE-HAMSTER HYBRID CELL LINES CARRYING THE neo-MINICHROMOSOME AND THE SAUSAGE CHROMOSOME AND COUNTAINING A COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME

G305

AND neo-

BrdU TREATMENT, SINGLE-CELL CLONING. SELECTION WITH HYGROMYCIN B

BrdU TREATMENT. SINGLE-CELL CLONING, SELECTION WITH G418, BrdU TREATMENT AND RECLONING

H₁D₃

MOUSE-HAMSTER HYBRID CELL LINE CARRYING A MEGACHROMOSOME BUT NO MINICHROMOSOME

FUSION WITH CD4+ HeLa CELLS CONTAINING neor. SELECTION WITH G418 AND HYGROMYCIN B

H1xHe41

MOUSE-HAMSTER-HUMAN HYBRID CELL LINE CARRYING THE MEGACHROMOSOME AND A SINGLE HUMAN CHROMOSOME WITH CD4 AND

GHB42 CARRIES MEGA-CHROMOSOME AND neo-MINICHROMOSOME

RECLONE AND GROW

MEGACHROMOSOME

MINICHROMOSOME

IN G418 AND

▼ HYGROMYCIN B

IN G418

RECLONE AND GROW

neo- MINICHROMOSOME ONLY

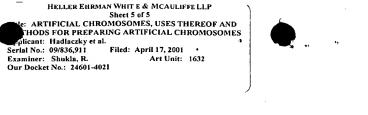
GB43

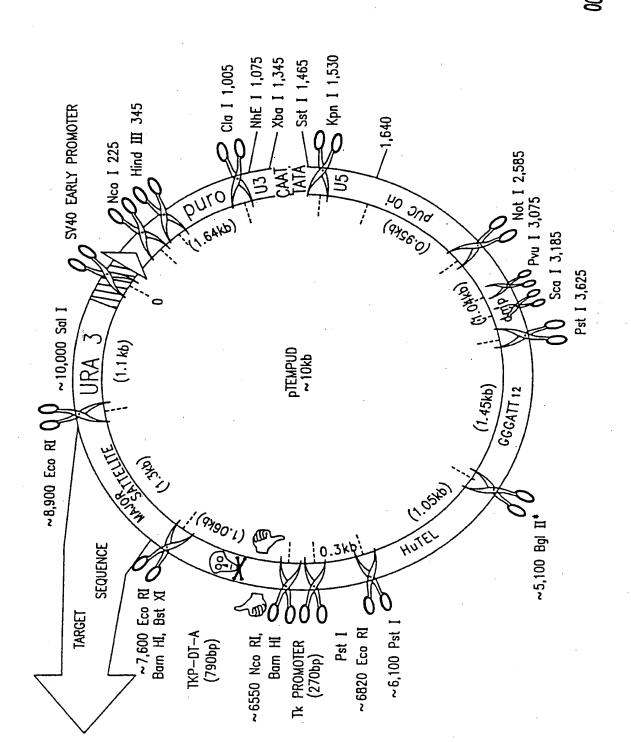
G3D6

MOUSE-HAMSTER HYBRID CELL LINES CARRYING:

CARRIES neo-MINICHROMOSOME ONLY

neor GENES; CONTAINS COMPLETE HAMSTER AND PARTIAL MOUSE GENOMES





-1G. 5